Technique for faecal marking in group-housed southern hairy-nosed wombats *Lasiorhinus latifrons*

L.A. Hogan[1,2*], C.J.C. Phillips[2], A.B. Horsup[3], T. Janssen[4] and S.D. Johnston[1]

[1]School of Animal Studies, The University of Queensland, Gatton Campus, Queensland Australia 4343
[2]Centre for Animal Welfare and Ethics, The University of Queensland, Gatton Campus, Queensland Australia 4343
[3]Environmental Protection Agency, Rockhampton, Queensland Australia 4701
[4]Rockhampton Botanical Gardens and Zoo, Rockhampton, Queensland Australia 4700

ABSTRACT

This study compared the efficacy of plastic glitter, with a mean particulate size of 1.18 mm, as a faecal marker in group-housed captive wombats *Lasiorhinus latifrons*. The wombats voluntarily consumed the glitter through the use of appetizing food vehicles. Over 40 different food treats were tested as possible vehicles for the oral delivery of the faecal marker and of these six were deemed highly palatable: (1) golden syrup with horse pellets, (2) golden syrup with weetbix, (3) pitted dates, (4) honey with kangaroo pellets, (5) nutrigel with rolled-oats, and (6) strawberry sauce with rolled-oats. Mean transit time of glitter particulates through the alimentary tract of *L. latifrons* was 2.9 ± 0.5 d, with maximal output occurring 4.2 ± 0.3 d after administration. A marker dose of 1.6 g / 3 d was required to reach a steady and detectable state of marker output. Using this dosage > 2 particulates (i.e. flecks) of glitter were defaecated in > 90% of faecal pellets, allowing the accurate identification of individual samples. Reliable labeling was obtained using gold, silver, metallic red, metallic green, metallic blue and white glitter, i.e. digestion did not affect the integrity of these colours. There was no evidence that long-term feeding of glitter had any negative effects on the normal formation of faecal pellets, the clinical health, weight or appetite of the wombats.

Key words: wombat; faecal marker; glitter; oral delivery

Introduction

The southern hairy-nosed wombat *Lasiorhinus latifrons* is currently held in 19 zoological institutions across Australia (ARAZPA 2009). *L. latifrons* are typically housed in small, mixed-sex social groups (i.e. one male with multiple females), to facilitate captive breeding. Faecal steroid analysis is a non-invasive method for monitoring changes in the concentration of reproductive- and stress-related hormone metabolites. However, it is only of value if there exists a way to reliably collect and identify individual samples. In group-housed animals, accurate identification of samples from individuals is difficult and time consuming, unless a faecal marker is incorporated into the diet.

A faecal marker is defined as a substance that is not much absorbed from the alimentary tract and may be almost completely recovered in the faeces (Kotb and Luckey 1972). They have been used in nutritional and metabolic studies to determine gastrointestinal transit times, dietary intake, feed digestibility, faecal output, dry matter absorbability and the efflux of water (Kotb and Luckey 1972; Warner 1981; Teeter and Owens 1983; Mandell *et al.* 1988; Adams *et al.* 1991; Chandler *et al.* 1997; Pendlebury *et al.* 2005). They have also been used to facilitate sampling in hormonal studies of grazing animals, i.e. to enable individual identification of faeces so that samples can be collected from the grazed sward areas (Kotb and Luckey 1972). Early markers included glass beads, small seeds, charcoal, cotton string, rubber, small pieces of metal and dyes (Kotb and Luckey 1972). Nowadays, plastic beads, raw corn and other poorly digestible grains, commercial food colourings and plastic glitter are the preferred markers for studies where individual identification of faeces is required (Moore and Traver 1978; Graham *et al.* 2000; Griffin 2002).

Ideally markers should be (1) inert with no toxic, physiological or psychological effects, (2) neither absorbed nor metabolized within the alimentary tract, (3) have no appreciable bulk, (4) mix intimately with the usual food and (5) have no influence on alimentary function (Kotb and Luckey 1972). Plastic beads have been shown to have an inconsistent passage (pyloricdiarrhoeamestic cats with continual use resulting in an increase in gastrointestinal acidity, vomiting and diarrhoea (Chandler *et al.* 1997). Raw corn and other hard-to-digest grains are metabolized in the alimentary tract of *L. latifrons*, as this species has a high dry-matter-content and neutral-detergent-fiber digestibility, due to a long retention time (52–62 h) of digesta (Barboza 1993). Commercial food-colourings are, in flavor, extremely bitter and it is difficult to disguise the taste of this food additive; as such it unpalatable to some animals (Griffin 2002). Conversely, plastic glitter has no taste, is not well absorbed by the alimentary tract, is non-toxic and free of harmful effects (Griffin 2002). The objectives of this study were to evaluate the use of plastic glitter as a faecal marker in captive *L. latifrons*, to identify palatable food treats for the oral delivery of the marker and to demonstrate the markers' safe and effective long-term use.

Methods

Animals

Twelve (4♂, 8♀) sexually mature (>8 yr) *L. latifrons*, housed at Rockhampton Zoo's research centre (23º23'S, 150º29'E) were utilized for this study. The animals were group housed (1♂:2♀) in four separate, adjacent enclosures. Each enclosure consisted of a temperature-controlled (air-conditioned) indoor area equipped with two sleeping dens and interrelated tunnels as well as an outdoor area with a soil and sand substrate, a digging chamber, a hollow log and trees (*Eucalyptus spp.*). Each outdoor area was partially vegetated with couch *Cynodon dactylon* and guinea grass *Panicum maximum*. Animals were fed daily with a mixture of kangaroo pellets (Riverina Australia Pty Ltd., Brisbane, Australia), oaten hay (Johnson and Sons, Kapunda, Australia), grass clumps (*Chloris spp., Austrostipa spp.*) and carrots. Water was available *ad libitum*. The mean body weight (± SE) of the male and female wombats at the start of the study was 27.3 ± 0.8 kg and 25.5 ± 1.8 kg, respectively. This study was approved by The University of Queensland Animal Ethics Committee.

Faecal marker

Plastic glitter was purchased from Sulyn Industries Incorporated (Coral Springs, Florida, USA) for use as a faecal marker. A multi-glitter sample was evaluated by ACTS Testing Labs Incorporated (Buffalo, NY, USA) and after toxicological review it was determined that the sample conformed to the Consumer Product Safety Commission guidelines (ASTM-D4236) and was non-toxic. Sieve analysis, using a nested column of sieves with wire mesh cloth (screen), was used to assess the particulate size distribution of the glitter. Analysis was conducted using a dry, tapping sieving technique. Particulate size distribution for a 15.2 g sample was 1.18–2.36 mm 0.03%, 0.06–1.18mm 90.0%, 0.04–0.06 8.4%, 0.03–0.04 0.09% and 0.01–0.03 0.03%.

Experimental design

Experiment 1: marker delivery vehicles

Forty-two food treats were tested from February 2005 to November 2005 as possible oral delivery vehicles for the marker. Each treat was offered to all 12 wombats, for five consecutive days, with a transitional period (i.e. a period where no treats were offered) of two days between each treat. In order to habituate the wombats to delivery, food treats (hereafter termed vehicles) were offered following a standardized procedure. The wombats were isolated prior to vehicle presentation in order to minimize food-related aggression and stealing. The vehicles were then presented in individually assigned food bowls, at the same time each afternoon (17:30 h) by the same food handler. The wombats were given their daily dietary rations after vehicle consumption, i.e. no food was available between 07:00- 19:00 h.

Vehicles consisted of a base (non-adhesive) ingredient, coated with a bonding (adhesive) ingredient containing 0.7 g of marker. Base ingredient portions were: 25 g rolled-oats (Woolworths Limited, NSW, Australia), 25 g kangaroo pellets (Riverina Australia Pty Ltd., QLD, Australia), 30 g horse pellets (Ridley Agriproducts, VIC, Australia), 10 g slice of wholemeal bread, 20 g weetbix (Sanitarium, NSW, Australia), 30 g sweet potato and 30 g carrots. Bonding ingredient portions were: 15 g molasses, 25 g orange marmalade (Cottee's, VIC, Australia), 15 g nutrigel (Troy Laboratories Pty Ltd., NSW, Australia), 20 g vegemite (Kraft Foods Ltd., VIC, Australia), 40 g peanut butter (Kraft Foods Ltd., VIC, Australia), 25 g strawberry jam (Woolworths Limited, NSW, Australia), 30 g honey (Woolworths Limited, NSW, Australia), 30 g strawberry sauce (Woolworths Limited, NSW, Australia), 30 g chocolate sauce (Woolworths Limited, NSW, Australia), 30 g caramel sauce (Woolworths Limited, NSW, Australia), 30 g golden syrup (CSR Australia, VIC, Australia), 40 g creamed corn (Woolworths Limited, NSW, Australia), 45 g cooked potato mash, 45 g cooked carrot mash, 30 g apple puree (HEINZ, VIC, Australia), 45 g avocado and 20 g pitted dates.

Vehicle palatability was determined from the amount consumed within 60 min after presentation and was classified as high if amount consumed was between 75-100% and low if amount consumed was < 75% (Griffin 2002). Consistency of vehicle intake was classified high if it was eaten ≥ 80% of the time and low if eaten < 80% of the time (Griffin 2002). Both vehicle palatability and intake consistency had to be classified as high in 4 / 5 testing days for the vehicle to be considered useful for the oral delivery of marker.

Experiment 2: marker dosage and colour

Six wombats (2♂ 4♀ were randomly selected to test the minimum amount of glitter necessary to adequately label their faecal pellets. Five marker quantities (0.7, 1.0, 1.3, 1.6 and 1.9 g) were tested from December 2005 to January 2006. Using the vehicles identified in Experiment 1, each wombat (n = 6) received a single dose of each of the marker quantities in random order. At the start, each wombat received their first randomly assigned marker dose. Two faecal samples (5 pellets / sample) from each wombat were collected per day (06:30 h; 19:30 h), for eight consecutive days, in order to monitor the output of glitter. Following a transitional period of two days, each wombat then received their second randomly assigned marker dose and again glitter output was monitored for eight days, followed by a transitional period of two days. This process repeated until all six wombats received a single dose of all five marker quantities. Marker output was quantified by a manual count of glitter particulates per pellet. Labeling consistency was based on the number of glitter particulates within each pellet as well as on the number of pellets containing glitter. Consistency was considered to be high if ≥ 2 glitter particulates were defaecated in ≥ 90% of the pellets and low if < 2 glitter particulates were defaecated in > 10% of the pellets (Griffin 2002).

In order to determine which glitter colours could be reliably used to label the wombats' faecal pellets, each wombat (n = 12) was randomly assigned a unique glitter colour for testing in February 2006. Colour allocation was: M1 gold, M2 silver, M3 metallic red, M4 cherry red, F1 metallic green, F2 forest green, F3 pink, F4 metallic blue, F5 sky blue, F6 yellow, F7 black and F8 white. Each wombat was fed their unique colour of glitter for one month, using a dosing schedule of 1.6 g / 3 d (dose determined from Part 1 of Experiment 2). To ensure accurate identification of samples, a human observer was used to pinpoint individual faecal deposits. Due to the manpower involved in this process, faecal sampling was tiered using a four day cycle: Day 1 samples were collected from the animals in Enclosure 1 (n = 3), Day 2 samples were collected from the animals in Enclosure 2 (n = 3), Day 3 samples were collected from the animals in Enclosure 3 (n = 3) and Day 4 samples were collected from the animals in Enclosure 4 (n = 3). This four day sampling cycle was then repeated (n = 7) for the entire month of February. As such, faecal samples (5 pellets / sample) were collected from each individual, every fours days and the defaecated glitter particulates were visually inspected. Colours that remained unaltered during transit through the alimentary tract were deemed suitable for use as a faecal marker.

Experiment 3: long-term marker use

The *L. latifrons* at Rockhampton Zoo were used for a non-invasive hormonal study from March 2006 to March 2007 (Hogan *et al.* 2010*a*; 2010*b*). During this experimental period, each wombat received an oral 1.6 g dose of marker every three days. Within each group, the male received metallic blue glitter, the smaller female received metallic red glitter, whilst the larger female received gold. A veterinary health check, on each animal, was performed prior to and immediately following the hormonal study. The veterinary health check included a complete physical examination, hematological and faecal evaluation. The wombats were also weighed fortnightly during the course of the study, to ensure proper weight maintenance.

Data analysis

Descriptive statistics were used to quantitatively summarize the data sets. Mean daily marker outputs, in terms of the number of (1) glitter particulates within each pellet and (2) pellets containing glitter, were calculated from an aggregation of data from all animals (n = 6) from each sampling day (n = 8) for every marker dose (n = 5). Repeated fortnightly body weight measurements were analyzed by two-way analysis of variance (ANOVA). Mean vehicle run-lengths, in terms of the length of time a vehicle was eaten before rejection, were calculated from a combination of data from all animals given that vehicle (n = 1-11) from all sampling days the vehicle was offered (n = 52-132) for each type of vehicle. Total mean vehicle run length was calculated as a mean of the mean vehicle run-lengths for all six vehicles.

Results

Experiment 1

Out of the 42 vehicles tested, six were useful for the oral delivery of marker. Vehicle palatability and intake consistency were high for (1) golden syrup with horse pellets in 11 wombats, (2) golden syrup with weetbix in seven wombats, (3) pitted dates in five wombats, (4) honey with kangaroo pellets in one wombat, (5) nutrigel with rolled-oats in one wombat and (6) strawberry sauce with rolled-oats in one wombat.

Experiment 2

The outputs of marker following a single dose administration of five differing quanities are presented in Figures 1 and 2. Mean transit time of glitter particulates through the alimentary tract was 2.9 ± 0.5 d, with maximum output occurring 4.2 ± 0.3 d after administration. Thereafter, marker output decreased so that by 8 d post-administration, marker was no longer evident in the faeces. Marker doses of 0.7, 1.0 and 1.3 g resulted in low labeling consistency. High labeling consistency, was achieved with both 1.6 and 1.9 g of glitter for three consecutive days (Days 4-6) after administration (Fig. 1 and 2).

Reliable labeling was obtained using gold, silver, metallic red, metallic green, metallic blue, white and silver glitter, i.e. digestion did not affect the integrity of these colours. The other colours were unsuitable because digestion faded and/or altered the original colour.

Experiment 3

From the results of Experiment 1 and 2 (i.e. observed marker transit time, dosage and period of high labeling consistency) it was deduced that the wombats required a marker dose of 1.6 g / 3 d in order to maintain a steady and detectable state of marker output. Using this dosing schedule, faecal pellets were consistently identifiable over a 13-month hormonal study. Clinical results indicated that there were no detectable adverse health effects from the long-term use of marker on body weight, hematology or faecal consistency. Live weight did not change significantly across the sampling period (*P* = 0.23), with the mean body weight (± SE) of the male and female wombats at the end of the study being 27.7 ± 1.0 and 26.3 ± 1.4 kg, respectively. Hematological tests revealed that sodium, potassium, chloride, glucose, urea, creatinine, calcium, phosphate,

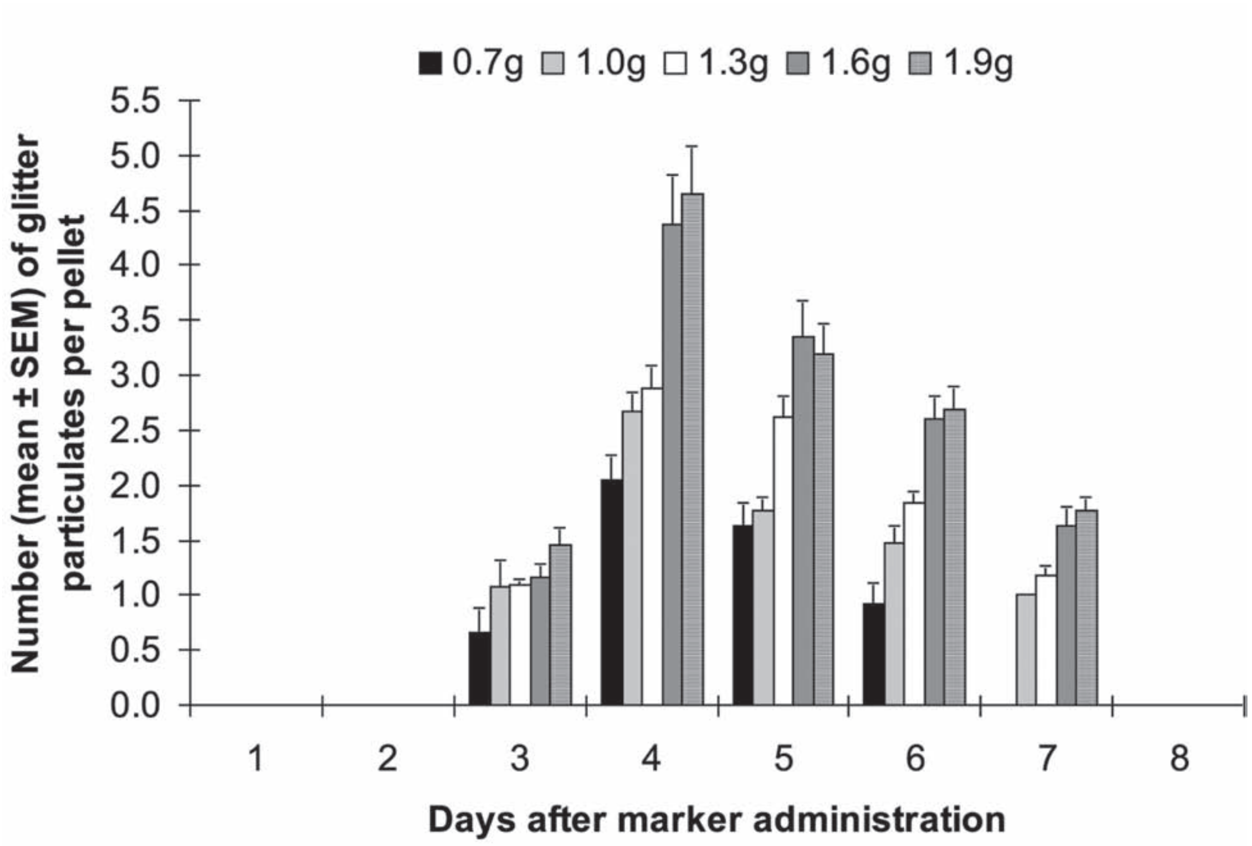

Figure 1. Output of glitter particulates (mean ± SEM, per pellet) in the faecal pellets of captive *L. latifrons* after administration of marker on Day 0.

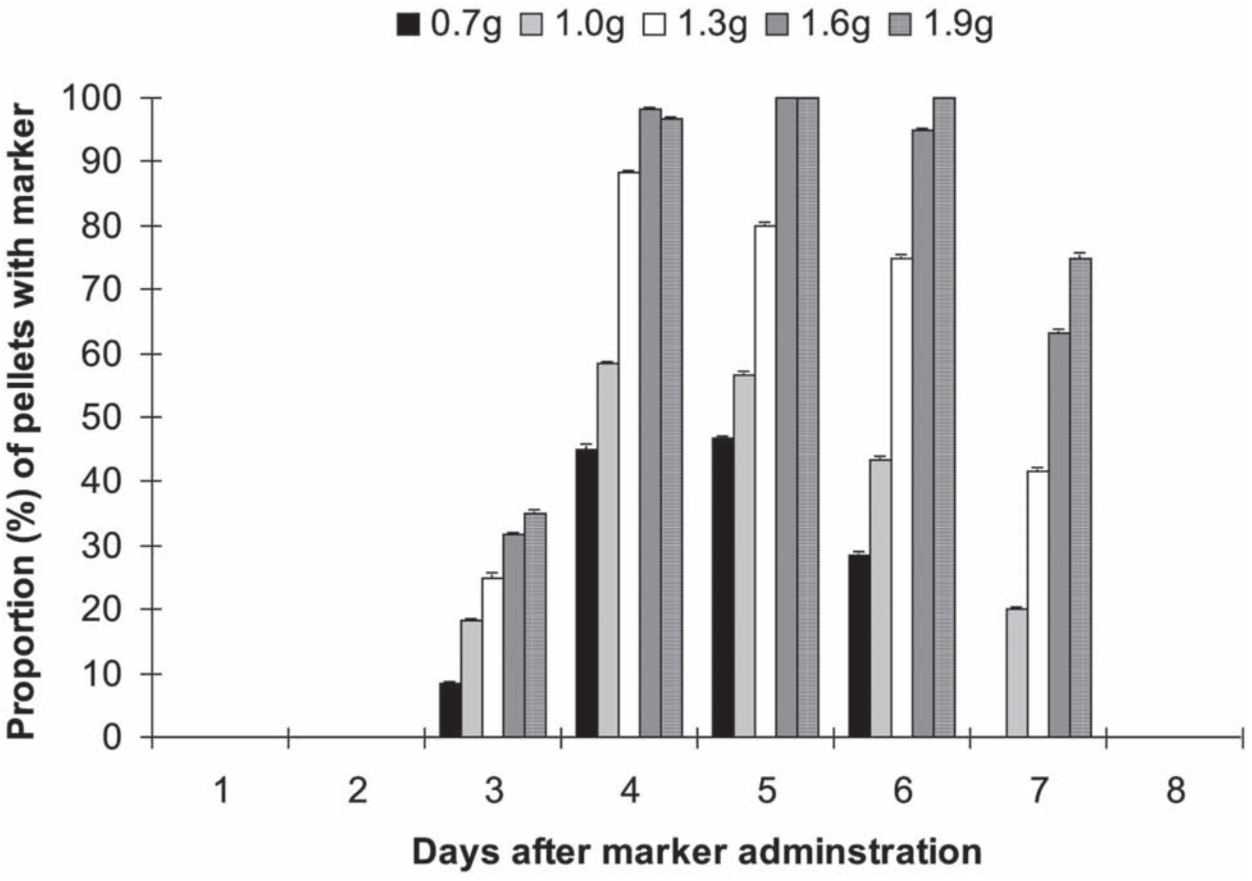

Figure 2. Proportion (%) of *L. latifrons* faecal pellets (mean ± SEM) containing marker after administration on Day 0.

protein, albumin, globulin, cholesterol, red and white blood cell count/morphology remained within normal ranges for *L. latifrons* (Clark 2004). Likewise, faecal pathology tests revealed that colour, consistency, starch, neutral fat, fatty acids, trypsin, occult blood and parasite burden values remained within normal ranges for *L. latifrons* (Nicolson 2007; *pers. comm.*).

Each wombat had a preference for two or more vehicles and these were interchanged whenever a wombat lost their appetite for one. Total vehicle run-length varied from a minimum of 13 d to a maximum of 252 d, with a mean length of 28.5 ± 4.7 d. Mean run-lengths for each vehicle were: 50.7 ± 9.9 d for golden syrup with horse pellets, 40.8 ± 9.3 d for golden syrup with weetbix, 23.7 ± 9.0 d for nutrigel with rolled-oats, 21.6 ± 2.4 d for pitted dates, 18.6 ± 4.5 d for strawberry sauce with rolled-oats and 15.0 ± 1.5 d for honey with kangaroo pellets.

Discussion

Plastic glitter, orally administered every three days, served as an effective faecal marker in *L. latifrons*. Griffin (2002) used milk products and canned cat food to facilitate the ingestion of glitter particulates in domestic cats. Similarly, in this study, oral administration of marker (i.e. glitter) was successfully achieved through the use of food vehicles; specifically golden syrup with horse pellets or weetbix, pitted dates, honey with kangaroo pellets and rolled-oats with nutrigel or strawberry sauce. The wombats did, however, lose their appetite for a vehicle when it was offered continuously. To ensure the long-term administration of marker it was necessary to alternate the vehicles when the appetite for one diminished. Vehicles were rotated on a regular basis, every 15-51 days depending on the wombat and the vehicle offered, so that the appetite for the marker-embedded food remained high. This result was different to that reported by Griffin (2002) who found that appetite for glittered gruel (1/8 tsp. plastic glitter in 15 mL canned evaporated milk) was long lasting, with 24 domestic cats eating this mixture daily over a period of 10 months. It is possible that the vehicles used within this study had a shorter appetizing time-span due to the fact that herbivorous wombats are likely to be less ‘food motivated’ in terms of food selection than carnivorous species.

The plastic glitter used in this study was classified useful as a faecal marker in *L. latifrons* as it did not appear to have any toxic or physiological effects (Kotb and Luckey 1972). Visual observation revealed that the glitter was not much absorbed by the alimentary tract, largely recoverable in the faeces and easily mixed into food vehicles. Clinical testing revealed that the glitter was non-toxic and had no effect on *L. latifrons* body condition, haematology or faecal pellet consistency. Optimum labeling results were obtained with gold, metallic red, metallic green and metallic blue coloured glitter. Cherry red, forest green, pink, sky blue, yellow and black coloured glitters were unsuitable because digestion faded and/or altered the original colour by (1) stripping the colour making the particulates appear white, clear or silver, or (2) tinting the particulates with a brown hue making them indistinguishable from other faecal components. Whilst digestion did not affect the integrity of the white and silver glitter, care needs to be taken in using these colours when monitoring multiple animals (i.e. when multiple colours are required) as digestion can, at times, partially remove the colour from some particulates, making them appear silver or white. Griffin (2002) also tested a variety of Sulyn Industries plastic glitters and found that five different colours were readily identifiable in the stools of domestic cats: red, green, pink, white and blue.

For dosing, a marker can be administered either as a single pulse dose, or it can be provided constantly for an extended period of time in an attempt to reach steady state conditions (Olivan *et al.* 2007). The goal of continuous or frequent dosing is to label the faeces uniformly with the marker. Dependable faecal labeling was obtained in this study by feeding 1.6 g of glitter, to each wombat, every three days. This dosing schedule resulted in high marker output (≥ 2 glitter particulates were defaecated in > 90% of pellets), enabling individual samples to be consistently and accurately identified. Faecal labeling did show some individual variability in the number of glitter particulates per pellet and defaecation surface, i.e. whether glitter was defaecated on the inside and/or outside surfaces of the pellet. Deviations in labeling could have been due in part to variations in the quantity and composition of food as well as individual differences in gut retention time, diet digestibility and faecal output (Olivan *et al.* 2007).

In summary this study has reported the successful development and application of a technique to mark (i.e. label) the faecal pellets of captive *L. latifrons*, thus enabling the accurate identification of individual samples in a group-housed setting. It is suspected that this technique, with minor modification, can be effectively applied to other herbivorous species. In addition, faecal marking also has a clinical application by enabling the quicker identification of an ailing wombat, within a multi-wombat environment.

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